nucleotide sequence. This nucleic acid differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations.

The method further includes providing a first oligonucleotide probe set, characterized by (a) a first oligonucleotide probe having a target specific portion and (b) a second oligonucleotide probe having a target-specific portion. The oligonucleotide probes in the first set are complementary to the first target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations. The probes are also suitable for ligation together when hybridized adjacent to one another on the first target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample. The method of the present invention also requires providing a second oligonucleotide probe set, characterized by (a) a third oligonucleotide probe having a target specific portion and (b) a fourth oligonucleotide probe having a target-specific portion. The oligonucleotide probes in the second set are complementary to the second target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations. The probes of the second set are suitable for ligation together when hybridized adjacent to one another on the second target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample.

The sample, the first and second oligonucleotide probe sets, and the thermostable ligase are blended together to form a ligase chain reaction mixture. The ligase chain reaction mixture is subjected to one or more ligase chain reaction cycles comprising a denaturation treatment and a hybridization treatment. During the denaturation treatment, any hybridized oligonucleotides are separated from the target nucleotide sequences. In the hybridization treatment, the oligonucleotide probe sets hybridize at adjacent positions in a base specific manner to their respective target nucleotide sequences, if present in the sample. The probes also ligate to one another to form a ligation product sequence containing the target specific portions connected together with the ligation product sequences for each set being distinguishable from other nucleic acids in the ligase chain reaction mixture. The oligonucleotide probe

sets may hybridize to nucleotide sequences in the sample other than their respective target nucleotide sequences but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment. The presence of ligation product sequences produced as a result of the target nucleotide sequence being present in the sample are then detected.

## EXAMPLES

### Example 1 - Reagents, Media, and Strains

All routine chemical reagents were purchased from Sigma Chemicals (St. Louis, MO) or Fisher Scientific (Fair Lawn, NJ). Restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Beverly, MA). Oligonucleotide synthesis reagents, DNA sequencing kits, and PCR kits were obtained from Applied Biosystems Division of Perkin-Elmer Corporation (Foster City, CA). dNTPs, BSA (i.e. bovine serum albumin), ATP were purchased from Boehringer-Mannheim (Indianapolis, IN). *Pfu* DNA polymerase was purchased from Stratagene (La Jolla, CA). *E. coli* strain NovaBlue(DE3)pLysS, and plasmid pET11c were purchased from Novagen, Inc. (Madison, WI). Protein assay kit was from Bio-Rad (Hercules, CA). HiTrap Blue affinity column was from Pharmacia (Piscataway, NJ). LB medium was prepared according to standard formula (Sambrook, et al., (1989) Molecular Cloning-A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1994), which is hereby incorporated by reference). Sonication buffer consisted of 50 mM Tris-HCl, pH 8.0 and 1 mM EDTA. TE buffer consisted of 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA. *Tth DNA* ligase and its mutant K294R were purified as previously described (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996), which is hereby incorporated by reference).

### Example 2 - Oligonucleotide Synthesis

Oligonucleotides were synthesized by using a 394 automated DNA synthesizer from Applied Biosystems Division of Perkin-Elmer Corp. PCR and sequencing primers were purified by ethanol precipitation according to instruction

manual. The degenerate sense primer 5'-ATC(T/A)(C/G)CGACGC(C/G)-GA(G/A)TA(T/C)GA-3' (SEQ. ID. No. 3) corresponding to amino acids 32-38 (ISDAEYD) (SEQ. ID. No. 4) in the *T. thermophilus* HB8 DNA ligase gene, and antisense primers 5'-CC(C/G)GT(C/G)C(G/T)-(G/C)CC(G/C)AC(C/T)TG(A/G)AA-3' (SEQ. ID. No. 5) and 5'-GCCTTCTC(C/G/A)A(A/G)(T/C)TTG-(C/G)(A/T)(G/C)CC-3' (SEQ. ID. No. 6) corresponding to amino acids 333-339 (FQVGRTG) (SEQ. ID. No. 7) and 641-647 (GSKLEKA) (SEQ. ID. No. 8) were used to amplify DNA ligase gene fragments from *Thermus* strains. Additional PCR and sequencing primers were synthesized as required. PCR amplification primers for cloning *Tsp.* AK16D DNA ligase gene into pET11c vector were 5'-GCGATTTCATATGACCCTAGAGGAGGCCCG-3' (SEQ. ID. No. 9) and 5'-GCGGGATCCGAGGC CTTGGAGAAGCTCTT-3', (SEQ. ID. No. 10) where the *Nde*I and *Bam*HI sites are underlined and the initiation codon in the forward primer is shown in bold. Oligonucleotide substrates for ligation assay were purified on a denaturing sequencing gel (7 M urea/10% polyacrylamide) (Applied Biosystems Inc., The complete guide to evaluating and isolating synthetic oligonucleotides, Applied Biosystems Inc., Foster City, CA (1992)). 5'-phosphorylation of oligonucleotides was achieved during synthesis by using Chemical Phosphorylation Reagent (Glen Research, Sterling, VA). Fluorescent group was attached to a 3'-terminus using Fluorescein CPG column (Glen Research).

**Example 3 - DNA Amplification, Cloning And Sequence Analysis**

Genomic DNAs from *Thermus* strains were isolated as previously described (Cao, et al., Gene, 197:205-214 (1997), which is hereby incorporated by reference). PCR amplifications with degenerate and unique primers and inverse PCR on circularized templates were carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems Division of Perkin Elmer) as described (Wetmur, et al., J Biol Chem, 269(41):25928-25935 (1994), which is hereby incorporated by reference). The nucleotide sequences of amplified ligase fragments were directly determined on an ABI 373 sequencer using ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer). Full length *Tsp.* AK16D DNA ligase gene was amplified using PCR amplification primers as described above,